CBC | CHICAGO BIOMEDICAL CONSORTIUM

CBC Catalyst Award Proposal Title Page

Title of the proposal *(no more than 100 characters including white space)*

Advanced microfluidic model of lung cancer for precision drug therapy

Name, degree, title, institution, and contact information including the email address of each PI

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Do you have any current or pending grant applications that potentially overlap with this application?
Check ONE:

YES ☐ **NO** ☒

If YES, please identify them.

(ENTER THE OVERLAP HERE)

Explain the overlap in the Biosketch section.

Does the proposed research involve animal subjects?	**Check ONE:**	**YES** ☐	**NO** ☒
Does the proposed research involve human subjects?	**Check ONE:**	**YES** ☐	**NO** ☒
Does the proposed research involve embryonic stem cells?	**Check ONE:**	**YES** ☐	**NO** ☒

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SUMMARY: Lung cancer is the leading cancer killer in the US. Targeted therapies can significantly improve the survival of lung cancer patients with targetable mutations, but the acquired resistance negatively impacts survival. Thus, a platform that uses minuscule amount of patient-derived cancer cells to identify clinically amendable drug therapies will significantly improve the patient survival. Unfortunately, limited progress has been made to address this need due to technical limitations. Our goal is to build a clinically amendable microfluidic platform for evaluating patient-specific drug response in lung cancer with 3D patient-derived tissues and the components of tumor microenvironment that influence drug response. We propose to use patient-derived organoids with oncogenic inhibitors to genetically delineate the tumor-microenvironment interaction, and evaluate drug response using a novel microfluidic platform. The proposed approach is transformative, and will ultimately permit patient-specific precision drug treatment plans, and to rapidly evaluate combinations of therapy.

HIGH-RISK/HIGH-REWARD: Currently, the diagnosis for druggable gene mutations and amplification in patient materials dominates precision medicine in NSCLC. We propose the clinically innovative concept that a microfluidic 3D culture platform will offer companion diagnostic information on whether the tumors are likely to respond to the targeted therapies. The microfluidic platform will permit to dynamically sculpt treatment profile, including combinations of inhibitors or chemotherapeutics, and escalation of their concentrations – which is not possible with current approaches. Use of only miniscule amounts of patient-derived materials will permit to extend to patient-specific tissues to guide development of individualized therapy plans in clinical and/or research settings. Our proposed device will permit to account for the impact of the tumor microenvironment (TME) in drug response, and will enable future studies of immune checkpoint inhibitors, inclusion of other TME components to recapitulate the complexity of human tumors, or inclusion of other *in vitro* organ models to model metastasis. Understanding the interactions of cancer cells and the TME is essential to successful development of immunotherapies. While potential of the proposed concept is tremendous, to fund it by the conventional sources (e.g., NIH), we need preliminary data and demonstration of feasibility.

PROPOSED RESEARCH:

Lung cancer is the leading cause of cancer-related deaths in the US[1] and the world.[2] Nearly 2/3^{rds} of patients are diagnosed at late stage with a 5-year survival rate of ~18%.[3] Many patients are diagnosed with non-small cell lung carcinoma (**NSCLC**) at non-operable late stage and thus receive therapies with targeted or chemotherapeutic agents. Targeted therapies can significantly improve survival in NSCLC patients harboring targetable mutations,[4,5] such as **EGFR**^{mut} or **KRAS** G12C^{mut}. Nevertheless, the emergence of *de novo* and acquired drug resistance continues to be an obstacle for the successful treatment of these patients. Thus, personalized models of human lung cancer are needed to determine effective drug combinations to overcome such resistance.

Limitation of patient-derived materials for personalized therapies: Fine needle aspirations are commonly used to monitor response to therapies and emergence of mutations, thus materials available for next generation sequencing and cell-based diagnostics for drug vulnerability is severely limited. To overcome the limitation, **PDOs** have been widely accepted as instructive 3D tumor models[6,7] that retain original tumor genotype, phenotype, and heterogeneity over passages. Thus, PDOs are ideal platforms for high-throughput drug screening and to simulate treatment response.[7]

Impact of the microenvironment on PDOs and the limitations of current methods: While PDOs are instrumental in simulating physiologically relevant cell-cell interactions, current *in vitro* NSCLC models do not account for the influence of the tumor microenvironment (**TME**) components on the survival of cancer cells. Yet, accumulating evidence shows that cellular and acellular components in TME influence how tumors respond to therapies.[8,9] These TME components include cancer-associated fibroblasts (**CAFs**), peripheral blood mononuclear cells (**PBMCs**), and extracellular matrix (**ECM**).[10] Understanding the interactions of cancer cells and the TME is essential to successful development of immunotherapies. To replicate TME *in vitro*, tumor, stromal, immune, endothelial, and other cells can be mixed and co-cultured in conventional cell culture dishes or transwell plates.[11-13] This approach however offers little control of the co-culture components. These methods are typical of 2D culture and do not fully recapitulate the 3D nature of tumors or lack the flow component present in TME *in vivo*.

Aims: We propose a microfluidic platform for *in vitro* modeling of NSCLC. We will use KRAS mutant

PDOs and recapitulate TME biology to evaluate patient-specific drug response. We also aim to assess TME interactions and their impact on drug response using transcriptomics and comparative gene expression analysis. Ultimately, we seek to formulate effective therapies to account for the factors derived from TME that affect drug response in NSCLC.

Aim 1: Demonstrate and optimize microfluidic platform for precision 3D models of NSCLC.

Rationale: Existing lung tumor platforms have multiple limitations, such as reliance on 2D monolayers that do not recapitulate *in vivo* 3D biology,[14,15] inconsistent size when 3D cultures are used, or mixed (tangled) co-cultures (with CAFs and PBMCs).

Methods: Here we overcome these shortcomings and aim to recapitulate TME-specific cancer growth. We will leverage our spheroid culture microwells, described in our (Shimamura & Papautsky) recent paper,[16] to develop and optimize a microfluidic device that allows 3D culture using 4 separate **PDOs** (CK7152, 8232, 2648, and 9121) with KRAS^{G12C} mutation from the NCI Repository and co-culture with CAFs from genotyped tumors from NSCLC patients and pooled PBMCs in separate but proximal compartments. First, the size of cancer cell spheroids is standardized via the uniquely shaped microwells, which helps the evaluation of anti-neoplastic agents more statistically significant. Second, each TME component (CAFs and PBMCs) can be joined to cancer cells in microwells, allowing them to interact in paracrine fashion. This will also enable us to analyze the cell-cell communication and delineate the impact of one another for the drug response. *Ultimately, the approach can be expanded to include other TME components or be applied to other disease and tissue types.*

Microfluidic platform: The microfluidic chip (**Fig. 1**) will consist of a microwell bottom layer and PDMS top layer in a form factor of a 3in x 1in microscope glass slide. The bottom layer (**Fig. 1B**) will contain ~100 U-shaped microwells per 400µm wide x 4cm long channel. Later iterations will increase the number of parallel channels up to 20 on a 3in square glass plate; we have built 50 parallel channel systems in the past. The microfluidic chip will arrange each of the TME cell types proximal to PDOs. To load 3 types independently, a **2nd layer** (75µm high channels) will be bonded on top of the chip to provide input/output channels (**purple lines** in **Fig. 1**), connected with vias. To load, PDOs/cells suspended in media will be flown into the appropriate input, and out via sink channel (**Fig. 1C**). Other inputs will be pressurized to permit flow to outlet and without crossing over. PDMS posts between chambers and sink channel (~5-10µm) will keep each tissue type in its own chamber. Flow rates and shear stress will be optimized to ensure high viability and function over 14-28d *in vitro*. During treatment, flow will be stopped, to permit paracrine signaling across static channel (**Fig. 1D**). Effluent will be collected for downstream analysis. Distance between PDOs and CAFs/PBMCs, determined by post width in the device, will be optimized from 400 to 50 µm.

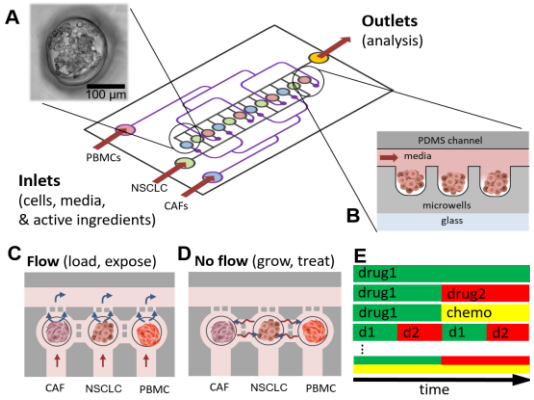

Figure 1. Microfluidic platform for 3D PDO co-culture with CAFs & PBMCs. (**A**) Device contains up to 20 parallel channels – only 1 shown schematically. **Purple lines** indicate channels in the 2nd (upper) layer. (**B**) PDMS channel layer is bonded to layer containing U-wells. Media and drug stimuli are perfused through microfluidic layer. (**C**) NSCLC PDO, CAF, and PBMC adjacent chambers with blocking posts allow cell loading and media change. Common channel used to collect media for analysis (or waste). (**D**) Flow is stopped to allow paracrine signaling. (**E**) **Sculpting** of treatment profiles enabled by the microfluidics platform.

Phenotype analysis. We will assess PDO size, shape (circularly), viability (Calcein-AM/PI), and expression of key oncogenes, total-KRAS, total-ERK, adherence (EpCAM), proliferation (MCM2), epithelial (E-cadherin), mesenchymal (N-cadherin) markers as we described previously.[16]

Co-culture: To delineate effects of CAFs and PBMCs in the drug resistance to targeted agents, purified cells will be used in microfluidic chip at various ratios (1:4, 1:1, 4:1 for CAFs/PBMCs to PDOs); supernatant from CAFs and PBMCs grown separately will also be used. Our preliminary results show that supernatant from WI-38 fibroblasts increases drug resistance of H358 KRAS^{G12C} cells.

Aim 2: Demonstrate reliable drug response of NSCLC models and assess TME interactions.

Rationale: We will iteratively use the microfluidic platform to quantitatively evaluate the pharmacodynamic characteristics in the presence of TME components with statistical significance, which is difficult with primary culture. Subsequent signaling and apoptotic pathwayanalyses together

with kinase and ligand profiling in Patient-Derived materials will give credence to clinically amendable microfluidic chips that help formulate therapeutic strategies.

Methods: Drug efficacy will be assessed using PDOs in microfluidic chip. We will use compounds relevant in the treatment in $KRAS^{mut}$ NSCLC for 48h or 72h. The microfluidic platform will permit us to dynamically sculpt treatment profile, including combinations of inhibitors or chemotherapeutics, and escalation of therapeutics' concentrations (**Fig. 1E**). Viability of PDOs will be assessed by imaging following treatment, with statistical evaluation at sufficient power ($p<0.05$) as described in our recent paper.[16] These studies will validate the microfluidic chip as a reliable platform for drug response. Viability of PDOs following drug treatment will also be assessed with or without the presence of CAFs and PBMCs. Following treatment, PDOs will be extracted from the microfluidic device by reverse flow, and lysed for pharmacodynamic studies evaluating apoptosis and signaling changes[17].

Single-cell transcriptomic analysis. RNA from NSCLC PDOs will be extracted pre and post treatment and analyzed via scRNA sequencing (**scRNAseq**). Microtissues will be extracted from the microfluidic device by reverse flow; cells will be harvested via collagenase as described previously.[18] *DropSeq* will be used for single cell analysis as described in our (Basu) paper.[19] If required number of cells is too great (>70k cells/analysis), a *10X Genomics* system capable of lower cell numbers (>2k cells/sample) but at higher cost will be used. Gene expression levels will be measured by TPM (transcripts per million reads). Comparative gene expression assessments will be done using the Seurat v3.0.2.[20] Differential expression analysis will be done using *FindMarkers* function, Wilcoxon Rank Sum test, and statistically significant genes will be extracted based on an adjusted p-value threshold of 0.05. To reduce cost, we will also explore pooling cells from multiple experiments using a combinatorial scheme for single cell RNA-seq runs; cells from different experiments will be demultiplexed back to their source experiments using natural genetic variation in patients.[21]

INTER-INSTITUTIONAL COLLABORATION: This application brings together investigators from two Chicagoland institutions, each with expertise critical to the project success. **Ian Papautsky** (UIC) is an expert in the development of microfluidic systems for precision medicine, including culture devices for modeling cancer, as well as devices for rare cell isolation, such as circulating tumor cells (CTCs) in liquid biopsy for cancer screening. **Takeshi Shimamura** (UIC) is an expert in the development of therapeutic strategies to overcome the acquired and *de novo* targeted-drug resistance using both *in vitro* and *in vivo* models in lung cancer. Papautsky and Shimamura developed agarose microwells for the 3D culture of NSCLC tumor spheroids with cell lines and PDOs that mitigate several limitations with current platforms such as inconsistent spheroid size and the presence of multiple spheroids per well.[16] **Anindita Basu** (U.Chicago) is an expert in the single-cell epi-genomic and transcriptomic profiling with applications in basic and translational research, and also develops tools to study inter-cellular interactions in complex biological systems. She has used single-cell transcriptomics to study TME in ovarian cancer using tissue biopsies.[19]

CRITERIA FOR SUCCESS: A) Growth curves and viability for PDOs matching "gold standard" dome cultures for >10d with $p<0.05$. **B)** Phenotype of each tissue model in the microfluidic device produces comparable (Pearson's $r>0.90$) or higher functions than controls. **C)** IC_{50} curves for each drug treatment highly correlated (Pearson's $r>0.95$) with known clinical response. **D)** Microfluidic platform faster (~5d) for drug screening with PDOs vs. ~3-4wks in "gold standard" dome culture. **E)** scRNA-seq yields 600-800 genes/cell and 1500 UMI/cell (UMI = universal molecular identifier), <40% mitochondrial RNA, and 75% of cells recovered in sequencing data.

FUNDING PLAN: A) NIH/NCI Innovative Molecular Analysis Technologies (IMAT) Program supports development of next-gen analytical methods in cancer research. We plan to target the R61 application in response to RFA-CA-22-001 (expected to be re-issued) for September 2023/April 2024. Depending on the research progress, we will target the advanced development R33 in response to RFA-CA-22-002 (expected to be re-issued) in September 2024/April 2025. **B)** NIGMS Technology Development Program supports technology development and prototype validation (PAR-22-127). We will target an R01 application in 2024.

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BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: **Papautsky, Ian**

eRA COMMONS USER NAME: **PAPAUTSKY**

POSITION TITLE: **Professor,** Biomedical Engineering, University of Illinois Chicago

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Boston University	BS	09/1995	Biomedical Engineering
University of Utah	PhD	12/1999	Bioengineering

A. Personal Statement

My research focus is on innovating microfluidic and sensing technologies for hematology, oncology, and precision medicine. My lab has pioneered the *inertial microfluidics* technology for label-free isolation and analysis of rare cells. Inertial microfluidics relies on interaction of hydrodynamic forces acting on flowing cells and leads to lateral migration across streamlines into specific cross-sectional positions depending on their size (*Lab Chip*, 13: 1121, 2013; *Microsystems & Nanoengineering*, 6: 105, 2020). The approach shows tremendous potential for high-throughput cell sorting in hematology (*Biomicrofluidics,* 7: 054101, 2013; *Scientific Reports*, 8: 9411, 2018). My lab has focused on liquid biopsy applications, including capture and molecular profile analysis of circulating tumor cells (CTCs) and circulating tumor microemboli (CTM), whose molecular profile can provide a "cancer census" that is more holistic representation of disease state and active pathophysiology (*Microsystems & Nanoengineering*, 5: 1-12, 2019; *Cancers* 11: 89, 2019).

As co-director of the NSF I/UCRC Center for Advanced Design & Manufacturing of Integrated Microfluidics (CADMIM), I developed a better understanding of the microfluidics technology needs, particularly for point-of-care and precision medicine applications. As part of the center activities, I interact with businesses in the areas of medical devices, pharma, public health, and precision agriculture, which have or are interested in developing products using microfluidics and biosensing technologies. Through these interactions, I have developed an appreciation for the pathways for technology commercialization, which is ultimately the critically-important step to translation of laboratory prototypes to the marketplace.

I have been fortunate to have opportunities to foster the development of undergraduates, graduate students, post-doctoral fellows, and junior faculty. I have mentored 4 postdoctoral fellows, 18 PhD as primary advisor, and numerous MS and undergraduate students. My laboratory provides an excellent environment for training in microfluidics and biosensors for trainees. My publications so far have been highly cited (>10,000 citations and h-index of 49 as of November 2022) and my lab has been continuously funded by NIH and/or NSF since 2002.

The overarching goal of this highly novel proposal with Dr. Takeshi Shimamura (MPI) at UIC and Dr. Oni Basu (MPI) at U.Chicago is to demonstrate microfluidic platforms for *in vitro* modeling of NSCLC that relies on patient-derived tissues, recapitulates TME biology, and permits to evaluate patient-specific drug response and to delineate TME impact using single-cell transcriptomics. The application leverages an existing collaboration with Dr. Shimamura, an expert in acquired and *de novo* targeted-drug resistance in NSCLC, and extend it to include Dr. Basu at U.Chicago, a world-leader in single-cell transcriptomics and developer of the *Drop-Seq* technology. The great working relationship and collaboration with Shimamura lab has led to a joint journal publication that forms the basis for the novel concept proposed in this application:

Q. Luan, J. Becker, C. Macaraniag, M. Massad, J. Zhou, **T. Shimamura,** & **I. Papautsky**. "Non-small cell lung cancer spheroid models in agarose microwells for drug response studies." *Lab Chip* 2022, **22**, 2364.

Ongoing Research Support:

NIH R33 ES024717 (PI: Papautsky, MPI: Haynes) 09/01/18-8/31/23

Validation and Demonstration of Point-of-Care Sensor for Multi-Metal Exposure Assessment
The goal of this project is to validate microfluidic point-of-care sensor technology for rapid and accurate measurement of blood manganese (Mn) and lead (Pb).
Role: PI

NSF IIP-1841473(PI: Papautsky) 3/1/19-2/28/24
Phase 2: I/UCRC for Advanced Design and Manufacturing of Integrated Microfluidics (CADMIM)
The major goal of this project is to continue the establishment of a national NSF center for microfluidic innovation.
Role: PI

NSF EECS-1917295 (PI: Xu) 5/1/19-4/30/23
Towards High-Throughput Label-Free Circulating Tumor Cell Separation using 3D Deterministic Dielectrophoresis (D3)
The goal of this project is to demonstrate a proof-of-concept microfluidic device concept that can be used for isolation of circulating tumor cells (CTCs) from blood.
Role: Co-PI

CADMIM Industrial Members (PI: Papautsky) 3/1/19-2/28/23
Projects funded by the industrial members of the NSF CADMIM Phase 2 center.
These are industrial projects funded by the industrial members of the NSF CADMIM Phase 2 I/UCRC at UIC. Projects are 1yr long. The project portfolio changes annually, with aims/objectives guided by the industrial advisory board.
Role: Co-PI

Completed Research Support:
NIH R01ES022933 (PI: Papautsky) 10/01/13-9/30/20
Development of a lab-on-a-chip for point-of-care biomonitoring of blood metals
The goal of this project was to develop a microfluidic "multi-metal chip" that will facilitate rapid and accurate measurement of blood manganese (Mn) and lead (Pb).
Role: PI

NSF IIP-1738617 (PI: Papautsky)
Phase 1: I/UCRC for Advanced Design and Manufacturing of Integrated Microfluidics (CADMIM)
The goal of this project is to support the establishment of a national NSF center for microfluidic innovation.
Role: PI

Univ. Illinois Cancer Center (PI: Hulbert)
Pilot Grant: Applied microfluidics for lung cancer liquid biopsy
The goal of this pilot project was to demonstrate liquid biopsy approach using inertial microfluidics for isolation of circulating tumor cells (CTS) and benchmark with cfDNA approaches.
Role: Co-I

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2018-	Member, University of Illinois Cancer Center, Chicago, IL
2016-	Richard and Loan Hill Professor, Department of Bioengineering, University of Illinois Chicago
2013-	Co-Director, NSF Center for Advanced Design and Manufacturing of Integrated Microfluidics
2013-2016	Professor, Department of Electrical and Computer Engineering & Department of Chemistry (secondary appointment), University of Cincinnati
2012–2016	Director, Ohio Center for Microfluidic Innovation (OCMI)
2008–2012	Director, University of Cincinnati Center for Micro/Nano Fabrication
2006–2010	Research Associate Professor, Department of Biomedical Engineering, University of Cincinnati
2006–2014	Associate Professor, Department of Electrical and Computer Engineering, University of Cincinnati
2002–2006	Research Assistant Professor, Department of Biomedical Engineering, University of Cincinnati
2000–2006	Assistant Professor, Department of Electrical and Computer Engineering, University of Cincinnati

Scientific Appointments & Honors

2020	Outstanding Editor of the Year, *Microsystems & Nanoengineerin*g (Nature Publishing Group)
2019	Editorial Advisory Board, *Lab on a Chip*, Royal Society of Chemistry (RSC)

2018 Fellow, American Institute for Medical and Biological Engineering (AIMBE)
2018 Departmental Established Research Faculty of the Year Award, College of Medicine, University of Illinois at Chicago, Chicago, IL
2017 Fellow, Royal Society of Chemistry (RSC)
2017 Richard and Loan Hill Professorship, Colleges of Medicine & Engineering, University of Illinois at Chicago, Chicago, IL
2016, 2009 Distinguished Engineering Researcher, College of Engineering, University of Cincinnati
2015 Dean Advisory Council, College of Engineering and Applied Sciences, Univ. Cincinnati
2013, 2004 William H. Middendorf Research Excellence Award, University of Cincinnati
2007 Ohio Bioscience 30 in Their 30s
2007 Master Engineering Educator, College of Engineering, University of Cincinnati
2007, 2005 Excellence and Service Award, International Society for Optical Engineering (SPIE)
2006, 2002 William E. Restenmeyer Teaching Excellence Award, University of Cincinnati
2003 Professor of the Quarter Award, College of Engineering, Univ. Cincinnati, Spring 2003

C. Contribution to Science

1) **Inertial microfluidics for label-free cell manipulation:** My recent work in microfluidics has focused on using hydrodynamic forces inside microfluidic channels to focus and separate cells. This approach is termed "inertial microfluidics". Inertial microfluidics is counterintuitive, and relies on interaction of shear and wall hydrodynamic forces acting on flowing cells. The result is lateral migration across streamlines into specific cross-sectional positions depending on their size. The approach shows tremendous potential for applications in hematology and cytometry, especially those requiring high-throughput or isolation of rare cells. The following publications describe the fundamentals of the approach, including detailed experimental investigations using high-speed imagining, as well as our recent review of the approach and its applications to cell sorting.

a. J. Zhou, I. Papautsky, "Resolving dynamics of inertial migration in straight and curved microchannels by direct cross-sectional imaging," *Biomicrofluidics*, 2021, 15: 014101.
b. J. Zhou, Z. Peng, I. Papautsky, "Mapping inertial migration in the cross section of a microfluidic channel with high-speed imaging," *Microsystems & Nanoengineering,* 2020, 6: 105.
c. J. Zhou and I. Papautsky, "Fundamentals of inertial focusing in microchannels" *Lab Chip,* 2013, 13: 1121.
d. J. Zhou, I. Papautsky, "Viscoelastic microfluidics: progress and challenges," *Microsystems & Nanoengineering,* 2020, 6 (1), 1-24.

2) **Isolation of CTCs:** My lab has been applying the inertial microfluidics approach to *hematology-oncology* applications, focusing on isolation of circulating tumor cells (CTCs) form blood in a liquid biopsy approach. We have demonstrated the possibility of a completely passive separation (i.e., no active external field or power) in low-cost, disposable microfluidic chips. Based on inertial microfluidic principles, the separation leverages differences in the size of CTCs (>15um dimeter) and blood cells (7-8 um diameter RBCs and 10-15um diameter WBCs). The quality (purity, efficiency) of separation is exceptionally high, >95%, with throughputs on the order of 2mL/min or 1million cells/min. In addition to capture and molecular profiling of individual CTCs from patients with lung, head & neck cancers, our approach has been successful in isolating circulating tumor microemboli/clusters. These efforts are discussed in the publications below, as well as our recent review on capture of cell clusters.

a. J. Zhou, A. Kulasinghe, A. Bogseth, K. O'Byrne, C. Punyadeera, and I. Papautsky, "Isolation of circulating tumor cells in non-small-cell-lung-cancer (NSCLC) patients using a multi-flow microfluidic channel", *Microsystems & Nanoengineering,* 2019, 5: 1-12.
b. A. Kulasinghe, J. Zhou, L. Kenny, I. Papautsky, and C. Punyadeera, "Capture of circulating tumour cell clusters using straight microfluidic chip," *Cancers*, 2019, 11: 89.
c. N. Nivedita, N. Garg, A. P. Lee and I. Papautsky, "A high throughput microfluidic platform for size-selective enrichment of cell populations in tissue and blood samples," *Analyst*, 2017, 142: 2558-2569.
d. Q. Luan, C. Macaraniag, J. Zhou, and I. Papautsky, "Microfluidic systems for hydrodynamic trapping of cells and clusters," *Biomicrofluidics*, 2020, 14: 031502.

3) **Blood fractionation:** In addition to applications in oncology, my lab has been applying inertial microfluidics to *hematology* and *cytometry*. This approach offers an ability to easily and efficiently separate RBCs and WBCs, or fractionate blood into cell sub-populations. The capability to focus cells into a single stream without requiring sheath flows permits cytometry for rare and fragile cells, which normally would not sustain shear due to sheath

flows. Further, our recent focus on inertial microfluidics with viscoelastic fluids offers a convenient way to isolate cells directly from whole blood, without any manipulation or dilution. This can be done in both spiral and straight microfluidic channels, as discussed in publications below.

a. J. Zhou and I. Papautsky, “Size-dependent enrichment of leukocytes from undiluted whole blood using shear-induced diffusion”, *Lab Chip,* 2019, 19: 3416-3426.
b. J. Zhou, C. Tu, Y. Liang, B. Huang, Y. Fang, X. Liang, I. Papautsky and X. Ye, “Isolation of cells from whole blood using shear-induced diffusion,” *Scientific Reports*, 2018, 8: 9411.
c. X. Wang, H. Gao, N. Dindic, N. Kaval, and I. Papautsky, “A low-cost, plug-and-play inertial microfluidic helical capillary device for high-throughput flow cytometry”, *Biomicrofluidics*, 2017, 11: 014107.
d. N. Nivedita and I. Papautsky, "Continuous separation of blood cells in spiral microfluidic devices," *Biomicrofluidics*, 2013, 7: 054101.

4) **Point-of-care electrochemical sensors:** My recent work in point-of-care sensors has focused on using electrochemical detection methods for determination of trace metals (namely Mn, Pb, Cd, Zn) in biological samples (whole blood, serum, urine) and water samples (surface, drinking). Both anodic and cathodic stripping voltammetry methods are used, in conjunction with miniature sensors that have a form-factor of a USB stick. In initial efforts, a bismuth (Bi) working electrode was used for metal determination. However, in effort to reduce cost of these disposable sensors, and increase their stability, a copper-based sensor (using a Cu working electrode and CuCl2 reference electrode) was developed. This innovative sensor approach was described in recent high-impact publications in journal *Analytical Chemistry*. The approach shows tremendous potential for rapid, low-cost, on-site determination of metals at very low limits of detection. The following publications describe the approach and its early applications to metal determination in biofluids.

a. E. Boselli, Z. Wu, A. Friedman, B. Claus Henn, and I. Papautsky, “Validation of electrochemical sensor for determination of manganese in drinking water,” *Environ. Sci. Technol.,* 2021, 55: 7501–7509.
b. Z. Zhang and I. Papautsky, “Miniature ion-selective electrodes with mesoporous carbon black as solid contact,” *Electroanalysis,* 2021. doi: 10.1002/elan.202100088
c. W. Kang, C. Rusinek, A. Bange, E. Haynes, W. R. Heineman, and I. Papautsky, “Determination of manganese using cathodic stripping voltammetry on a platinum thin-film electrode”, *Electroanalysis*, 2017, 29, 686.
d. W. Kang, X. Pei, A. Bange, W. R. Heineman, I. Papautsky, “Copper-based electrochemical sensor with palladium electrode for cathodic stripping voltammetry of manganese,” *Anal. Chem.*, 2014, 86: 12070.

5) **Point-of-care calorimetric sensors:** In addition to the sensor work described above, my lab has used optical detection methods for calorimetric and fluorescence based sensing applications. In both cases, we used low-cost complimentary metal-oxide-semiconductor (CMOS) array image detector to measure light. In a CMOS array detector each pixel contains four photodetectors, of which two are covered by green, one by red, and one by blue band-pass (Bayer) filters. Thus, images taken by a CMOS detector consist of three color channels: red, green and blue (RGB). The CMOS detector will offer ease in alignment and low power consumption. For fluorescence lab-on-a-chip systems, while semiconductor LEDs were used as portable light sources. In these systems, filtering of excitation light from signal light is critical due to their stacked arrangement. My lab pioneered the use of cross-polarization for filtering the excitation and emission light. This approach was used successfully in fluorescence-based imaging of O_2 and CO_2 and for calorimetric assay quantification.

a. R. C. Murdock, K. M. Gallegos, J. A. Hagen, N. Kelley-Loughnane, A. Weiss, and I. Papautsky, “Development of a point-of-care diagnostic for influenza detection with antiviral treatment effectiveness indication,” *Lab Chip,* 2017, 17: 332 332.
b. L. Shen, J. Hagen, I. Papautsky, “Point-of-care colorimetric detection with a smartphone,” *Lab Chip*, 2012, 12: 4240–4243.
c. R. Murdock, L. Shen, D. Griffin, N. Kelley-Loughnane, I. Papautsky, J. Hagen, “Optimization of a paper-based ELISA for a human performance biomarker,” *Anal. Chem.*, 2013, 85: 11634.
d. L. Shen, M. Ratterman, D. Klotzkin, and I. Papautsky, “A CMOS optical detection system for point-of-care chemical sensors,” *Sensors & Actuators: B. Chemical*, 2011, 155: 430-435.

List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/ian.papautsky.1/bibliography/public/

BIOGRAPHICAL SKETCH

NAME: **Takeshi Shimamura, Ph.D.**

eRA COMMONS USER NAME (credential, e.g., agency login): **TSHIMAMURA2516**

POSITION TITLE: **Associate Professor**

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Western Michigan University, MI U.S.	B.S.	12/1994	Biology/Chemistry
Western Michigan University, MI U.S.	M.S.	04/1998	Immunology/Virology
Western Michigan University, MI U.S.	Ph.D.	12/2002	Molecular Biology
Schepens Eye Research, Harvard Medical School, MA U.S.	Postdoc.	01/2003	Molecular Biology
Dana-Farber Cancer Institute, Harvard Medical School, MA U.S.	Postdoc.	02/2008	Cancer Cell Biology

A. Personal Statement

I am a tenured associate professor in the Department of Surgery and University of Illinois Cancer Center at University of Illinois Chicago (UIC). My laboratory focuses on understanding the mechanisms that drive acquired targeted-drug resistance with cancer cell intrinsic factors and the interplay between cancer cells and the tumor microenvironment (TME) including tumor associated fibroblast (TAF), tumor associated blood vessels, and tumor-associated macrophages (TAMs) in non-small cell lung carcinoma (NSCLC). Our goal is to develop therapeutic strategies to overcome the acquired and *de novo* drug resistance in NSCLC.

I have broad background in cancer cellular and molecular biology, with extensive expertise in signal transduction and developmental therapeutics. I have been working on NSCLC, mesenchymal cells, TME, and cancer associated vasculatures for 20 years. Through these studies, I have established the experimental platforms and gained the substantial expertise and leadership skills necessary to successfully carry out new research programs. As a postdoctoral fellow at Dana-Farber Cancer Institute (DFCI), my original discovery that EGFR mutation-positive NSCLC are exquisitely sensitive to HSP90 inhibition evolved into a project in the DFCI/Harvard Cancer Center Lung SPORE, sponsored by NCI, and has also been translated into clinical trials. With support from the American Cancer Society, we have discovered that the inhibition of mutant EGFR-positive NSCLC with EGFR TKIs results in the release of TGFβ1 that is preferentially used by a subpopulation with a mesenchymal phenotype for survival and heterogeneity within EGFR mutant NSCLC cells gives rise to divergent resistance mechanisms in response to anti-TGFβ1 treatment (Cancer Research 2015 & 2016). We reported that CXCR7, an atypical G-protein coupled receptor (GPCR), is necessary and sufficient for the survival of the resistant cells and maintenance of a mesenchymal phenotype and concurrent EGFR and CXCR7 inhibition in the resistant cells resulted in mesenchymal to epithelial transition and cell death (Cancer Research 2019). This discovery led us to NIH RO1 grant and the recruitment to University of Illinois Chicago as a tenured associate professor. With the realization that tumor vasculature in TME is an important therapeutic target, our research culminated in the discovery that EGFR TKI resistant NSCLC secretes endothelin-1 to constrict tumor feeding blood vessels to reduce drug penetration in the tumors (Cancer Research 2020). We have extended our expertise in acquired drug resistance to investigate the biology of EGFR and KRAS G12C inhibitors acquired resistance using Patient-Derived Organoid (PDO) models. We forged a collaboration with Dr. Ian Papautsky in the Department of Bioengineering at UIC to develop microfluidic microwell system to study drug responses in NSCLC cells and published our preliminary work (Lab on a Chip 2022) laying ground for utilizing primary patient specimens to formulate personalized NSCLC treatment.

In summary, I have a demonstrated record of productive research publications and successful research projects in an area of high relevance for NSCLC patients. My broad research experience, scientific expertise, and inspired leadership as well as active collaborations with other groups have prepared me to successfully accomplish the objectives of the proposed research project.

The most relevant publication:

1. Pulido I, Ollosi S, Aparisi S, Becker JH, Aliena-Valero A, Benet M, Rodriguez ML, Lopez A, Tamayo-Torres E, Chulia-Peris L, Garcia-Canaveras JC, Soucheray M, Dalheim AV, Salom JB, Qiu W, Kaja S, Fernandez-Coronado JA, Alandes S, Alcacer J, Al-Shahrour F, Borgia JA, Juan O, Nishimura MI, Lahoz A, Carretero J, **Shimamura T***. Endothelin-1-Mediated Drug Resistance in EGFR-Mutant Non-Small Cell Lung Carcinoma. **Cancer Res**. 2020;80(19):4224-32. PMCID: PMC7541638.

2. Luan Q, Becker JH, Macaraniag C, Massad MG, Zhou J, **Shimamura T**, Papautsky I. Non-small cell lung carcinoma spheroid models in agarose microwells for drug response studies. **Lab Chip**. 2022;22(12):2364-75. Epub 20220614.

Ongoing and recently completed research support:

Ongoing research support:

RO1CA230778 (PI: Shimamura) 8/5/19 – 7/31/24
NIH/NCI
Investigation of CXCR7 signaling in EGFR TKI resistant NSCLC
The major goals of this project are to delineate the mechanisms of CXCR7 activation, signal transduction, and transcriptional activation leading to acquired drug resistance in EGFR mutant NSCLC.

LCRP Concept Award W81XWH2210023 (PI: Pulido – postdoctoral fellow) 1/10/22 – 1/09/23
Department of Defense
Metabolic vulnerabilities associated to G12C inhibitors resistance
The major goals of this project are to investigate metabolic vulnerability by targeting NNMT activation in KRAS G12C inhibitor resistant NSCLC.

LCRP Concept Award (PI: Shimamura) 2/1/23 – 1/31/24
Department of Defense
"Improve the Efficacy of Lung Cancer Therapy by Targeting the EDN1 Axis"
The major goals of this project are to investigate the roles of EDN1, a major vasoconstrictor, to limit the flow of drug carrying blood stream to the tumors following the treatment with targeted agents.

Completed Research Support (within the last 3 years)**:**

Research Scholar Grant 126638-RSG-14-229-01-TBG 1/1/15 – 12/31/19
American Cancer Society
LKB1 modulates MAPK pathway to control MYC expression in mutant KRAS NSCLC
The major goals of this project are to investigate the impact of LKB1 mutation on regulating MYC expressing and dependency for the therapeutic resistance in KRAS mutant NSCLC.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

05/2019-Present	Associate Professor (with tenure), Department of Surgery, College of Medicine, UI Cancer Center, University of Illinois at Chicago, Chicago, IL
11/2011-04/2019	Assistant Professor, Oncology Research Institute, Department of Molecular Pharmacology, Loyola University Chicago, Maywood, IL
07/2008-10/2011	Instructor in Medicine, Dana-Farber Cancer Institute, Harvard Medical School, Boston MA

Scientific Appointments

2021 – Current	President, American Society for Pharmacology and Experimental Therapeutics (ASPET), Great Lakes Chapter.
2021 – Current	American Cancer Society, Cancer Cell Biology Peer Review Committee
2021 – Current	NCI Immuno-Oncology Research – ZRG1 OTC-D (08)
2018 – Current	Associate Editor, BMC Pulmonary Medicine. Topic Editor, Frontiers in Pharmacology
2017 – 2018	State of Pennsylvania, Department of Health Lung Cancer Research Program Reviewer
2016, 2017	DOD CDMRP Lung Cancer Research Program (LCRP) Scientific Reviewer
2016 – Current	State of Florida Department of Health Lung Cancer Research Program Reviewer
2014 – 2021	Ad Hoc Reviewer, NCI Special Emphasis Panel/Scientific Review Group STTR/SBIR,
2011 – Current	Scientific Advisory Board Member, Free ME from Lung Cancer, State of Maine Lung Cancer Research Foundation (http://www.freemefromlungcancer.org/)

2008 – Current, Ad-hoc scientific reviewer for the following journals: All AACR journals, Nature Communications, Journal of Clinical Investigation, Journal of Pharmacological Sciences, Scientific Reports, Lung Cancer, Expert Review of Molecular Diagnostics, British Journal of Pharmacology, Oncogene, Cancers, Oncotarget.

1995 – Current Memberships in AAAS, Sigma Xi, AACR, and ASPET

Honors

2019 Honorable Mention Basic Research Award, EGFR Resisters Research Summit.

2018 Outstanding Service Award, American Society for Pharmacology and Experimental Therapeutics (ASPET), Great Lakes Chapter.

2015 American Cancer Society Research Scholar Award

2010 Dana-Faber/Harvard Cancer Center, Lung Cancer Career Development Award

2008 Dana-Farber Cancer Institute, Claudia Adams Barr Award for Cancer Research

2005 Dana-Faber/Harvard Cancer Center, Lung Cancer Career Development Award

C. Contributions to Science

1. Investigated strategies to overcome acquired EGFR TKI resistance in NSCLC.

EGFR tyrosine kinase inhibitors (TKIs) are highly effective frontline therapeutics commonly used in combating NSCLC with EGFR kinase domain mutations but become less efficient with the emergence of secondary *EGFR* mutations or bypass pathway activations. Consequently, developing treatment strategies to overcome acquired EGFR TKI resistance in NSCLC patients is clinically significant. A mesenchymal phenotype is clinically associated with acquired EGFR TKI resistance in NSCLC patients with unknown resistance mechanisms. My laboratory uncovered the mechanisms underlying the etiology of EGFR TKI resistance of NSCLC featuring a mesenchymal phenotype and demonstrated that preventing the emergence of mesenchymal subpopulation by inhibiting the TGF β pathway inadvertently select for a rare subpopulation of cells capable of triggering alternative resistance mechanisms (Cancer Res, 2015 and 2016). Our laboratory also reported that CXCR7 promotes a mesenchymal phenotype in EGFR TKI resistant tumors and the inhibition of CXCR7 sensitizes the resistant cells to EGFR TKIs (Cancer Res 2019). In August 2020, we reported in Cancer Research that EGFR TKI resistant NSCLC secretes endothelin-1 to constrict tumor feeding blood vessels to reduce drug penetration in the tumors. This discovery is the foundation of this application. In collaboration, we are developing microfluidic microwell chip to evaluate NSCLC 3-D spheroids (Lab Chip 2022) and NSCLC PDOs.

a. Soucheray M, Capelletti M, Pulido I, Kuang Y, Paweletz CP, Becker JH, Kikuchi E, Xu C, Patel TB, Al-Shahrour F, Carretero J, Wong KK, Janne PA, Shapiro GI**, Shimamura T**. Intratumoral Heterogeneity in EGFR-Mutant NSCLC Results in Divergent Resistance Mechanisms in Response to EGFR Tyrosine Kinase Inhibition. Cancer Res. 2015;75(20):4372-83. PMCID: 4548796.
b. Becker JH, Gao Y, Soucheray M, Pulido I, Kikuchi E, Rodriguez ML, Gandhi R, Lafuente-Sanchis A, Aupi M, Alcacer Fernandez-Coronado J, Martin-Martorell P, Cremades A, Galbis-Caravajal JM, Alcacer J, Christensen CL, Simms P, Hess A, Asahina H, Kahle MP, Al-Shahrour F, Borgia JA, Lahoz A, Insa A, Juan O, Janne PA, Wong KK, Carretero J, **Shimamura T**. CXCR7 Reactivates ERK Signaling to Promote Resistance to EGFR Kinase Inhibitors in NSCLC. Cancer Res. 2019;79(17):4439-52. PMCID: 6746175.
c. Pulido I, Ollosi S, Aparisi S, Becker JH, Aliena-Valero A, Benet M, Rodriguez ML, Lopez A, Tamayo-Torres E, Chulia-Peris L, Garcia-Canaveras JC, Soucheray M, Dalheim AV, Salom JB, Qiu W, Kaja S, Fernandez-Coronado JA, Alandes S, Alcacer J, Al-Shahrour F, Borgia JA, Juan O, Nishimura MI, Lahoz A, Carretero J, **Shimamura T**. Endothelin-1-Mediated Drug Resistance in EGFR-Mutant Non-Small Cell Lung Carcinoma. Cancer Res. 2020;80(19):4224-32. PMCID: 7541638.
d. Luan Q, Becker JH, Macaraniag C, Massad MG, Zhou J, **Shimamura T**, Papautsky I. Non-small cell lung carcinoma spheroid models in agarose microwells for drug response studies. Lab Chip. 2022;22(12):2364-75. Epub 20220614.

2. HSP90 inhibition to overcome acquired EGFR TKI resistance in NSCLC.

I have contributed to the discovery of the therapeutic potential of Hsp90 inhibition in NSCLC, where I identified that NSCLC cell lines harboring epidermal growth factor receptor (EGFR) mutations are exquisitely sensitive to HSP90 inhibitors. I have demonstrated that Hsp90 inhibitor and EGFR irreversible inhibitor combined with rapamycin could overcome the acquired resistance mediated by the emergence of T790M. These findings have evolved into a full project in the Dana-Farber/Harvard Cancer Center Lung specialized program of research excellence (SPORE), sponsored by NCI and have also been translated into HSP90 clinical trials in NSCLC.

a. **Shimamura T,** Lowell AM, Engelman JA, Shapiro GI. Epidermal growth factor receptors harboring kinase domain mutations associate with the heat shock protein 90 chaperone and are destabilized following exposure to geldanamycins. Cancer research. 2005;65(14):6401-8.
b. **Shimamura T,** Li D, Ji H, Haringsma HJ, Liniker E, Borgman CL, Lowell AM, Minami Y, McNamara K, Perera SA, Zaghlul S, Thomas RK, Greulich H, Kobayashi S, Chirieac LR, Padera RF, Kubo S, Takahashi M, Tenen DG, Meyerson M, Wong KK, Shapiro GI. Hsp90 inhibition suppresses mutant EGFR-T790M signaling and overcomes kinase inhibitor resistance. Cancer research. 2008;68(14):5827-38. PMCID: 3272303.
c. **Shimamura T**, Shapiro GI. Heat shock protein 90 inhibition in lung cancer. J Thorac Oncol. 2008;3(6 Suppl 2):S152-9. PMCID: 18520302.
d. **Shimamura T**, Perera SA, Foley KP, Sang J, Rodig SJ, Inoue T, Chen L, Li D, Carretero J, Li YC, Sinha P, Carey CD, Borgman CL, Jimenez JP, Meyerson M, Ying W, Barsoum J, Wong KK, Shapiro GI. Ganetespib (STA-9090), a nongeldanamycin HSP90 inhibitor, has potent antitumor activity in in vitro and in vivo models of non-small cell lung cancer. Clin Cancer Res. 2012;18(18):4973-85. PMCID: 3477583.

3. Investigating the biology of HER-family receptors with Kinase activating mutations in NSCLC.

The discovery of EGFR with kinase domain mutations and their sensitivity to EGFR TKIs significantly advanced targeted therapies for NSCLC patients. The thoracic oncology group at Dana-Farber Cancer Institute is one of the groups that co-discovered EGFR mutation and I was being trained as a postdoctoral fellow at the time of the landmark discovery. My publications represent a significant body of work that has contributed to the field of signaling mediated by mutant epidermal growth factor receptor (EGFR) and mutant HER2 receptors in NSCLC. Using the first model of murine lung adenocarcinoma induced by mutant EGFR that also harbors the T790M mutation, I reported that that the combination of an irreversible EGFR and an mTOR inhibitor is synergistic in cells with kinase domain/T790M compound mutations *in vitro*, translating to dramatic responses preclinically *in vivo*. These results have stimulated a clinical trial combining an irreversible EGFR inhibitor with an mTOR inhibitor in lung cancer

a. Ji H, Li D, Chen L, **Shimamura T**, Kobayashi S, McNamara K, Mahmood U, Mitchell A, Sun Y, Al-Hashem R, Chirieac LR, Padera R, Bronson RT, Kim W, Janne PA, Shapiro GI, Tenen D, Johnson BE, Weissleder R, Sharpless NE, Wong KK. The impact of human EGFR kinase domain mutations on lung tumorigenesis and in vivo sensitivity to EGFR-targeted therapies. Cancer Cell. 2006;9(6):485-95.
b. **Shimamura T,** Ji H, Minami Y, Thomas RK, Lowell AM, Shah K, Greulich H, Glatt KA, Meyerson M, Shapiro GI, Wong KK. Non-small-cell lung cancer and Ba/F3 transformed cells harboring the ERBB2 G776insV_G/C mutation are sensitive to the dual-specific epidermal growth factor receptor and ERBB2 inhibitor HKI-272. Cancer research. 2006;66(13):6487-91. PubMed PMID: 16818618.
c. Li D*****, **Shimamura T***, Ji H, Chen L, Haringsma HJ, McNamara K, Liang MC, Perera SA, Zaghlul S, Borgman CL, Kubo S, Takahashi M, Sun Y, Chirieac LR, Padera RF, Lindeman NI, Janne PA, Thomas RK, Meyerson ML, Eck MJ, Engelman JA, Shapiro GI, Wong KK. Bronchial and peripheral murine lung carcinomas induced by T790M-L858R mutant EGFR respond to HKI-272 and rapamycin combination therapy. Cancer Cell. 2007;12(1):81-93. **(*Equal Contribution)**
d. Deng J, **Shimamura T**, Perera S, Carlson NE, Cai D, Shapiro GI, Wong KK, Letai A. Proapoptotic BH3-only BCL-2 family protein BIM connects death signaling from epidermal growth factor receptor inhibition to the mitochondrion. Cancer research. 2007;67(24):11867-75.

4. Characterization of new therapeutic approaches in cancer using novel *in vitro* and *in vivo* models.

Lung cancer is a leading cause of cancer death in the U.S. NSCLC patients are often diagnosed at advanced stages when surgical removal of the tumor is not an option. Consequently, deep understanding of tumor biology and subsequent development of novel therapeutic approaches benefits NSCLC patients. In collaboration with other lung and breast cancer research laboratories, we have contributed in exploring new approaches in cancer treatment. The study of HSP27 in angiogenesis is a relevant work for this application.

a. Li D, Ambrogio L, **Shimamura T**, Kubo S, Takahashi M, Chirieac LR, Padera RF, Shapiro GI, Baum A, Himmelsbach F, Rettig WJ, Meyerson M, Solca F, Greulich H, Wong KK. BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models. Oncogene. 2008;27(34):4702-11. PMCID: PMC2748240.
b. Straume O, **Shimamura T**, Lampa MJ, Carretero J, Oyan AM, Jia D, Borgman CL, Soucheray M, Downing SR, Short SM, Kang SY, Wang S, Chen L, Collett K, Bachmann I, Wong KK, Shapiro GI, Kalland KH, Folkman J, Watnick RS, Akslen LA, Naumov GN. Suppression of heat shock protein 27 induces long-term

dormancy in human breast cancer. Proc Natl Acad Sci U S A. 2012;109(22):8699-704. PMCID: PMC 3365195.

c. Akbay EA, Koyama S, Carretero J, Altabef A, Tchaicha JH, Christensen CL, Mikse OR, Cherniack AD, Beauchamp EM, Pugh TJ, Wilkerson MD, Fecci PE, Butaney M, Reibel JB, Soucheray M, Cohoon TJ, Janne PA, Meyerson M, Hayes DN, Shapiro GI, **Shimamura T**, Sholl LM, Rodig SJ, Freeman GJ, Hammerman PS, Dranoff G, Wong KK. Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. Cancer Discov. 2013;3(12):1355-63. PMCID: PMC 3864135.
d. Christensen CL, Kwiatkowski N, Abraham BJ, Carretero J, Al-Shahrour F, Zhang T, Chipumuro E, Herter-Sprie GS, Akbay EA, Altabef A, Zhang J, **Shimamura T**, Capelletti M, Reibel JB, Cavanaugh JD, Gao P, Liu Y, Michaelsen SR, Poulsen HS, Aref AR, Barbie DA, Bradner JE, George RE, Gray NS, Young RA, Wong KK. Targeting Transcriptional Addictions in Small Cell Lung Cancer with a Covalent CDK7 Inhibitor. Cancer Cell. 2014;26(6):909-22. PMCID: PMC 4261156.

5. Investigating Epithelial to Mesenchymal Transition (EMT) and drug resistance in NSCLC with mutant KRAS and LKB1.

I played a key role in demonstrating that mice bearing lung cancers with activating *kras* mutations survive longer than mice with tumors harboring activated KRAS and concomitant loss of the LKB1 tumor suppressor protein. In the study, we also identified LKB1 as a critical tumor suppressor for pulmonary tumorigenesis that regulates initiation, differentiation and metastasis. Dr. Carretero and I have extended the finding by analyzing primary and metastatic *de novo* murine *kras*^{G12D} and *kras*^{G12D}/lkb1$^{-/-}$ lung tumors with integrated genomic and proteomic profiles. We demonstrated that the metastatic lung tumors exhibit EMT phenotype and are sensitive to concomitant treatment with SRC, PI3-K, and MEK1/2 inhibitors. In collaboration with Dr. Kwok-Kin Wong's laboratory at DFCI, my laboratory has also demonstrated that LKB1 loss in mutant KRAS NSCLC is a biomarker of BET bromodomain inhibitor, JQ1.

a. Ji H, Ramsey MR, Hayes DN, Fan C, McNamara K, Kozlowski P, Torrice C, Wu MC, **Shimamura T**, Perera SA, Liang MC, Cai D, Naumov GN, Bao L, Contreras CM, Li D, Chen L, Krishnamurthy J, Koivunen J, Chirieac LR, Padera RF, Bronson RT, Lindeman NI, Christiani DC, Lin X, Shapiro GI, Janne PA, Johnson BE, Meyerson M, Kwiatkowski DJ, Castrillon DH, Bardeesy N, Sharpless NE, Wong KK. LKB1 modulates lung cancer differentiation and metastasis. Nature. 2007;448(7155):807-10.
b. Carretero J*, **Shimamura T***, Rikova K, Jackson AL, Wilkerson MD, Borgman CL, Buttarazzi MS, Sanofsky BA, McNamara KL, Brandstetter KA, Walton ZE, Gu TL, Silva JC, Crosby K, Shapiro GI, Maira SM, Ji H, Castrillon DH, Kim CF, Garcia-Echeverria C, Bardeesy N, Sharpless NE, Hayes ND, Kim WY, Engelman JA, Wong KK. Integrative genomic and proteomic analyses identify targets for Lkb1-deficient metastatic lung tumors. Cancer Cell. 2010;17(6):547-59. PMCID: 2901842. **(*Equal Contribution**)
c. Chen Z, Cheng K, Walton Z, Wang Y, Ebi H, **Shimamura T**, Liu Y, Tupper T, Ouyang J, Li J, Gao P, Woo MS, Xu C, Yanagita M, Altabef A, Wang S, Lee C, Nakada Y, Pena CG, Sun Y, Franchetti Y, Yao C, Saur A, Cameron MD, Nishino M, Hayes DN, Wilkerson MD, Roberts PJ, Lee CB, Bardeesy N, Butaney M, Chirieac LR, Costa DB, Jackman D, Sharpless NE, Castrillon DH, Demetri GD, Janne PA, Pandolfi PP, Cantley LC, Kung AL, Engelman JA, Wong KK. A murine lung cancer co-clinical trial identifies genetic modifiers of therapeutic response. Nature. 2012;483(7391):613-7. PMCID: 3385933.
d. **Shimamura T***, Chen Z, Soucheray M, Carretero J, Kikuchi E, Tchaicha JH, Gao Y, Cheng KA, Cohoon TJ, Qi J, Akbay E, Kimmelman AC, Kung AL, Bradner JE*, Wong KK*. Efficacy of BET bromodomain inhibition in Kras-mutant non-small cell lung cancer. Clin Cancer Res. 2013;19(22):6183-92. PMCID: 3838895. **(*Corresponding Authors**)

Full list of my published work (49 total) is available at:
http://www.ncbi.nlm.nih.gov/sites/myncbi/takeshi.shimamura.1/bibliography/47248351/public/?sort=date&direction=descending

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: **Basu, Anindita**

eRA COMMONS USER NAME: **ANINDITABASU**, ORCID: 0000-0001-9468-3727

POSITION TITLE: **Assistant Professor**, Genetic Medicine, University of Chicago

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Arkansas	B.S.	04/2000	Physics, Computer Engineering
University of Pennsylvania	Ph. D.	07/2012	Physics
Harvard University, Broad Institute	Postdoctoral	11/2016	Systems Biology, Applied Physics

A. Personal Statement

I do research and teach in multi-disciplinary science at the intersection of genomics, medicine, microfluidics and nano/bio materials. I aim to bring new tools to aid in diagnosis, understanding and treatment of disease. I trained in soft matter Physics during my PhD, followed by post-doctoral studies in Applied Physics and Systems Biology. My lab develops and uses high throughput single-cell genomics to map different organs in health and disease, including human heart, gut and female reproductive system and fish fins.

Microbial cells are integral to health and well-being, and under certain conditions, can cause disease. I work to understand the interplay of genetics, immune system and the microbiome in inflammatory bowel disease. I am developing a suite of microfluidic and microscopy tools to profile host-pathogen interactions in the gut environment at single cell resolution, and for single microbial cell RNA-seq.

In a qualitatively different project, I am combining medical imaging, 3D modeling, 3D printing and soft lithography to build personalized fluidic models that capture a patient's unique vein geometry, hemodynamics, and bio-chemical factors. These patient-specific models are used to predict repeated thrombosis and stenosis events in patients undergoing regular hemodialysis.

Since establishing my independent research lab five years ago, I have been training and mentoring four graduate students and seven post-docs. Of them, one graduate student and three post-docs have obtained research positions in industry and pharma after completing their training in my lab. I have also trained eight undergraduate students and six post-baccalaureate researchers, almost all of whom continue to graduate programs in Biology or Public Health. My lab also hosts undergraduate students for summer research experience.

Ongoing Research Support

1DP2AI158157-01 (Basu) 08/20/2020-03/31/2025
NIH
Profiling Transcriptional Heterogeneity in Microbial Cells at Single Cell resolution and High-throughput using Droplet Microfluidics
Progress in single microbial cell genomics has been hindered due to challenges in 1) sheer variety of microbial taxa, species and strain-specific biological properties, 2) lysis of tough cell walls, and 3) low signal from relatively sparse microbial mRNA. I propose to combine recent developments in single cell genomics with new lysis and sequencing schemes to develop a generalized single-cell microbial RNA-seq pipeline that will allow us to process ~10^4-10^5 cells and capture ~500 transcripts per cell at reduced reagent and sequencing costs.
Role: PI

5RC2DK122394-03 (Chang) 09/01/2019-05/31/2024
NIH

Host and microbial basis of human ulcerative colitis and pouchitis: Identification, role, mechanisms, and resource development of host susceptibility and pathobiont factors
This proposal will take a novel and transformative approach to identify both disease susceptibility and microbial triggers that are involved in IBD, using the lens of the human ulcerative colitis (UC) model where patients have undergone total proctocolectomy with ileal pouch anal anastomosis (IPAA).
Role: Co-I

1R01HL147571-01 (Moskowitz) 06/01/2019-05/31/2023
NIH/NHLBI
The molecular basis of cardiac differentiation control
The goal is to investigate the novel hypothesis that loss of Hedgehog signaling causes premature cardiac progenitor differentiation as an underlying cause of CHD. If correct, this work will highlight molecular control of differentiation timing as a cornerstone of cardiac development.
Role: Co-I

R-1903-03784 (Basu) 08/15/2019-08/14/2023
Helmsley Charitable Trust
A Cell Atlas of the ileal colonic Crohn's Disease
The goal is to identify and characterize cell types in the human gut by establishing a comprehensive cellular atlas of the ileum in healthy individuals and patients with ileal-colonic and ileal Crohn's disease.
Role: PI

CZF2019-002435 (Lengyel) 07/01/2019-06/30/2023
Chan Zuckerberg Initiative
A Female Reproductive Cell Atlas
Characterize all cell types in the healthy female human reproductive tract using single cell RNA and ATAC sequencing comparing tissue from pre- and postmenopausal women integrating genomic and epigenetic information
Role: Co-I

Completed Research Support

5R01HL092206-10 (Gilad) 04/15/2009-04/30/2022
NIH/NHLBI
eQTL mapping in iPSC-derived differentiated cardiomyocytes
The goals are to characterize regulatory robustness in single-cell gene expression data from differentiated cardiomyocytes, identify genomic loci in which genetic variation is associated with inter-individual differences in regulatory noise (robustness QTLs), and use them to identify novel loci that are associated with CVD risk.
Role: Co-I

1R21AI144417-01 (Basu) 01/10/2019-12/31/2020
NIH/NIAID
Novel microfluidic platform to profile host-pathogen interaction under controlled infection and single cell resolution
To build droplet microfluidic and molecular biology techniques to study host-pathogen interaction in controlled microenvironment using imaging and single-cell genomics. This investigation will enable us to decipher the role of genetic heterogeneity in infection outcome, as well as identify virulent subsets in pathogen population

BSF Research Award (Basu) 08/01/2018 – 07/31/2020
U.S. – Israel Binational Science Foundation
Single Cell Gene Expression Analysis of Matrix-Directed Mesenchymal Stem Cell Differentiation
To profile genetic expression patterns of Stem Cell differentiation under mechanical cues using Drop-seq.
Role: PI

FP071803-01-PR (Basu) 12/01/2018 – 11/30/2019
Bristol-Myers Squibb
Unbiased expression profiling of cellular sub-populations using high-throughput droplet mRNA-seq for immunotherapy biomarker discovery in archival solid tumor samples
To develop a high-throughput droplet mRNA-seq protocol to map the solid tumor micro-environment, with emphasis on immune-infiltrate cells formalin-fixed, using paraffin embedded tumor samples.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2001-2003	Tutor, Physics, Chemistry, Math, Statistics, Student Support Services, University of Arkansas, AR
Spring, 2003	Research Assistant, Physics, University of Arkansas, AR
Summer, 2003, 2004	Research Intern, Physics, IBM- T.J. Watson Research Center, NY
2004-2005	Teaching Assistant, Physics, University of Pennsylvania, PA
2005-2012	Research Assistant, Physics, University of Pennsylvania, PA
Summers, 2005-2011	Laboratory Instructor, Material Science, University of Pennsylvania, PA
2012-2016	Post-doctoral Researcher, Applied Physics, Harvard University; Systems Biology, Broad Institute, MA
2016-2020	Assistant Scientist, Center for Nanoscale, Materials, Argonne National Laboratory, IL
2016-Present	Assistant Professor, Genetic Medicine, University of Chicago, IL

Scientific Appointments and Professional Memberships

2001-2004	Member, Society of Physics Students, University of Arkansas, Fayetteville chapter
2003-2004	President, Society of Physics Students, University of Arkansas, Fayetteville chapter
2009-	Member, American Physical Society
2017-	Member, American Association for the Advancement of Science
2010-	Journal Reviewer, Soft Matter, Journal of Fluorescence, Journal of Nanoparticle Research, Langmuir, Cell, Genome Biology, Nature Biotech
2015	Discussion leader, Soft Condensed Matter Physics, Gordon Research Seminar
2017-	Member, American Association for the Advancement of Science
2019-	Reviewer for NIH 'Cell and Molecular Technologies' study section

Honors

2000-2004	Chancellor's Scholarship, University of Arkansas
2000-2004	Dean's list, College of Engineering, University of Arkansas
2003	Financial award, US Particle Accelerator School
2003	American Physical Society/IBM grant
2003-2004	Richardson Scholarship, University of Arkansas
2004	Lingelbach Award, University of Arkansas
2005	Financial award, US Particle Accelerator School
2018	Kavli Fellow, National Academy of Sciences
2018, 2019	Scialog Fellow of Research Corporation for Scientific Advancement & Gordon and Betty Moore Foundation
2020	NIH Director's New Innovator Award

B. Contribution to Science

1. Develop and benchmark droplet microfluidic technology for single cell transcriptome analyses

I co-developed 'Drop-Seq', an innovative, high-throughput technology to profile the transcriptome of single mammalian cells at massively high throughput and low cost. This technology enables us to create a detailed taxonomy of cells from complex tissues based on mRNA expression levels in tens of thousands of single cells in a single experiment from which unbiased genomic classifications of cell types and/or cell-states can be developed. Applying this technique for a comparative taxonomy of healthy vs. diseased cells also help us map the cellular sites of action from which physiological deficits and/or malfunctions arise. Since its publication, Drop-seq was adopted in labs across the world, and it featured in the NIH Director's blog in 2015.

Though Drop-seq functions well for suspension cells and easily dissociable tissues, cell damage arising from tissue dissociation process may be a limiting factor for certain tissue-types like brain, heart, and solid tumors. Additionally, Drop-seq often fails to work for frozen tissue. I co-developed an alternate method that profiles single *nuclei* harvested directly from solid tissue: Droplet single nuclei mRNA-seq or DroNc-seq. This method robustly produces RNA libraries from single nuclei, including newly transcribed RNAs as well as long non- coding RNAs, and was successfully used to profile cell-types in hippocampus and pre-frontal cortex in frozen and archived human brain tissue, a system that was not accessible to single cell genomics before.

My lab benchmarked and compared the efficacy of high throughput single-cell and single-nucleus RNA-seq using Drop-seq and DroNc-seq. We established that DroNc-seq can be used as effectively as Drop-seq for cell type clustering and lineage differentiation on human iPSC derived cardiomyocytes. We then applied DoNc-seq to profile cellular composition of *post mortem* adult heart tissue that is not amenable for Drop-seq.

1) E. Z. Macosko, **A. Basu**, R. Satija, J. Nemesh, K. Shekhar, M. Goldman, I. Tirosh, A. R. Bialas, N. Kamitaki, E. M. Martersteck, J. J. Trombetta, D. A. Weitz, J. R. Sanes, A. K. Shalek, A. Regev, and S. A. McCarroll, *Highly Parallel Genome-Wide Expression Profiling of Individual Cells Using Nanoliter Droplets*. Cell, 161, 1202 (2015). PMCID: PMC4481139

2) N. Habib*, I. Avraham-Davidi*, **A. Basu***, T. Burks, K. Shekhar, M. Hofree, S. R. Choudhury, F. Aguet, E. Gelfand, K. Ardlie, D. A. Weitz, O. Rozenblatt-Rosen, F. Zhang and A. Regev, *Massively parallel single nucleus RNA-seq with DroNc-seq*. Nature Methods*,* 14, 955 (2017). PMCID: PMC5623139

3) A. Selewa, R. Dohn, H. Eckart, S. Lozano, B. Xie, E. Gauchat, R. Elorbany, K. Rhodes, J. Burnett, Y. Gilad, S. Pott, **A. Basu**, *Systematic Comparison of High-throughput Single-Cell and Single-Nucleus Transcriptomes during Cardiomyocyte Differentiation*. Scientific Reports 10(1), 1535 (2020). PMCID: PMC6992778

4) *A droplet-based method and apparatus for composite single-cell nucleic acid analysis*, WO 2016040476

2. Apply single cell genomics to decipher cellular heterogeneity in different organs and organisms

We are using single cell genomic techniques to characterize the cellular compositions and functions in diverse biological systems. We are characterizing the cellular composition of healthy adult human organs according to their transcriptomic and epigenomic signatures as part of the global *Human Cell Atlas* initiative. Toward this end, we are profiling the gut, female reproductive system and heart. As contrast, we are also studying some common diseases associated with these organs, like inflammatory bowel disease and ovarian cancer.

We are also working on increasing the sensitivity of Drop-seq and DroNc-seq using microfluidics, molecular biology and bioinformatics. This allows us to perform high-throughput single cell profiling of systems that are not amenable to standard Drop-seq or DroNc-seq procedures otherwise due to challenges like low RNA quality, quantity or difficulty with cell lysis.

1) A. Guzzetta, M. Koska, M. Rowton, K. R. Sullivan, J. Jacobs-Li, J. Kweon, H. Hidalgo, H. Eckart, A. D. Hoffmann, R. Back, S. Lozano, A. M. Moon, **A. Basu**, M. Bressan, S. Pott, I. P. Moskowitz, *Hedgehog-FGF signaling axis patterns anterior mesoderm during gastrulation.* Proc Natl Acad Sci 117(27):15712 (2020). PMCID: PMC7354932

2) S. Olalekan, B. Xie, R. Back, H. Eckart, **A. Basu**, *Characterizing the tumor microenvironment of metastatic ovarian cancer by single cell transcriptomics.* Cell Reports 35, 109165 (2021). PMID: 34038734

3) E. Lengyel, Y. Li, M. Weigert, L. Zhu, H. Eckart, M. Javellana, S. Ackroyd, J. Xiao, S. Olalekan, D. Glass, S. Iyer, A. J. Bilecz, R. Lastra, M. Chen, **A. Basu**, *A molecular atlas of the human postmenopausal fallopian tube and ovary from single-cell RNA and ATAC sequencing*. Cell Reports, accepted

3. Develop hybrid nanofabricated microfluidic (Bio-Nano) devices and techniques for single microbial cell transcriptomics

High-throughput scRNA-seq has been very successful in profiling the single cell transcriptomes of higher, multicellular organisms, but the technology has severely lagged for use with single-cell microbial life. Variable, rigid cell walls and magnitudes less mRNA present in a cell are some outstanding technical challenges. To deal with these complicating factors, we are developing a suite of fluidic and MEMS-based devices and techniques to be able to profile single microbial cells at throughput. We are also working to

- Extend Drop-Seq to study host-pathogen interaction at controlled multiplicity of infection and profile their paired transcriptomes based on outcome
- Develop different tissue-like biopolymer substrates with tunable physical and chemical properties
- Probe effects of chemical stimuli under tunable concentrations on cellular differentiation and growth

1) R. Dohn, B. Xie, R. Back, A. Selewa, H. Eckart, R. Prusty Rao, **A. Basu**, *mDrop-seq: Massively parallel single-cell RNA-seq of Saccharomyces cerevisiae and Candida albicans.* Vaccines, **2022**, 10, 30. PMID: 35062691

2) *High throughput dynamic reagent delivery system*, WO 2017075549

3) Microfluidic and MEMS cell lysis system and method UCHI: 21-T-034 (Pending)

4. Model hemodynamics and risk of thrombosis and stenosis in ESRD patients undergoing dialysis

The current gold standard for End-Stage Renal Disease (ESRD) patients undergoing dialysis is the creation of a surgically grafted arteriovenous fistula that allows a mature vein to withstand frequent dialysis sessions. Unfortunately, a vast majority of patients undergoing dialysis suffer AVF thrombosis, primarily in the cephalic vein arch. Understanding why cephalic arch thrombosis is common in ESRD patients is challenging due to the complex interplay of flow dynamics, biochemical factors in blood and activation of the vein's endothelium. We are building patient-specific fluidic models of the cephalic arch in ESRD patients that recapitulate the geometry and flow dynamics. Multi-factorial flow experiments using flow parameters, patient blood, anti-coagulants, thinners, chemical assays, etc. permit evaluation of their role in thrombosis and stenosis. Elucidating different thrombogenic factors will allow efficient, personalized treatments to pre-empt or treat complications.

1) M. Hammes, A. Moya-Rodriguez, C. Bernstein, S. Nathan, R. Navuluri, **A. Basu**, *Computational modeling of the cephalic arch predicts hemodynamic profiles in patients with brachiocephalic fistula access receiving hemodialysis*. PLoS ONE, 16(7), e0254016 (2021). DOI: 10.1371/journal.pone.0254016

2) 20. A. Moya-Rodríguez, B. Xie, D. Cook, M. Klineberg, S. Nathan, M. Hammes, **A. Basu**, *Creating Patient-Specific Vein Models to Characterize Wall Shear Stress in Hemodialysis Population*. Computational and Structural Biotechnology Journal, 20, 5729 (2022)

3) *Millifluidic system for thrombosis analysis under patient-specific physiological conditions*, US 2021/0056867

5. Rheology of soft gels and colloids

For my PhD research, I examined the validity of the affine transformation assumption under shear deformation in both flexible and semi-flexible polymer gels. This was done by embedding fluorescent tracer beads within the polymer networks and quantifying their displacements under a globally applied shear deformation. I built a customized setup for this purpose that coupled a rheometer with a confocal microscope. Gels studied include polyacrylamide, fibrin and collagen gels. The effects of strain, persistence length, and polymer and cross-link concentrations on non-affinity were systematically explored. The gels were studied under a wide range of applied strain, well into the strain-stiffening regimes for semi-flexible polymers, using Large Amplitude Oscillatory Shear (LAOS) analysis. I found that non-affine behavior increased with persistence length and decreased with mesh-size, according to current polymer elasticity theory.

I also performed a qualitatively different set of measurements to explore the rheology of monodisperse and bidisperse colloidal suspensions across the jamming transition. The experiments employed soft, temperature- sensitive microgel spheres for easy tuning of sample volume fraction in order to explore scaling behaviors of shear stress versus strain rate, and storage/loss shear moduli versus frequency. Under steady shear, the macroscopic measurements exhibit predicted scaling behavior for volume fractions above and below jamming. I also found that the storage and loss moduli of jammed systems, measured as functions of oscillatory frequency and volume fraction, can be scaled onto two distinct master curves, as suggested by simulation.

1) Q. Wen, **A. Basu**, J. Winer, A. Yodh, P. Janmey, *Local and global deformations in a strain-stiffening fibrin gel*. New Journal of Physics 9, 428 (2007). DOI: 10.1088/1367-2630/9/11/428

2) **A. Basu**, Q. Wen, X. Mao, T. C. Lubensky, P.A. Janmey, and A. G. Yodh, *Non-affine displacements in flexible polymer networks*. Macromolecules 44, 1671 (2011). DOI: 10.1021/ma1026803

3) Q. Wen, **A. Basu**, P. A. Janmey, and A. G. Yodh, *Non-affine deformations in polymer hydrogels.* Soft Matter 8, 8039 (2012). PMCID: PMC3445422

4) **A. Basu***, Y. Xu*, T. Still, P. E. Arratia, Z. Zhang, K. N. Nordstrom, J. M. Rieser, J. Gollub, D.J. Durian and A. G. Yodh, *Rheology of Soft Colloids Across the Onset of Rigidity: Scaling Behavior, Thermal, and Non-thermal Responses.* Soft Matter 10, 3027 (2014). PMID: 24695615

A complete list of publications may be found at NIH My Bibliography
https://www.ncbi.nlm.nih.gov/myncbi/anindita.basu.1/bibliography/public/

Program Director/Principal Investigator (Last, First, Middle): Papautsky, Ian

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY	FROM	THROUGH
	6/1/23	5/31/25

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Papautsky, Ian	PD/PI	0.24			203,700	4,074	1,448	5,522
Shimamura, Takeshi	MPI	0.24			160,000	3,200	1,138	4,338
Zhou, Jian	post-doc	4			64,500	21,500	7,640	29,140
Pulido, Ines	post-doc	4			64,500	21,500	7,640	29,140
SUBTOTALS →						50,274	17,866	68,140

CONSULTANT COSTS
n/a

EQUIPMENT *(Itemize)*
n/a

SUPPLIES *(Itemize by category)*
see next page page

12,860

TRAVEL
$1,000 per PI for travel to conferences — 1,000

INPATIENT CARE COSTS

OUTPATIENT CARE COSTS

ALTERATIONS AND RENOVATIONS *(Itemize by category)*
n/a

OTHER EXPENSES *(Itemize by category)*
$500/yr is requested for expenses associated with publishing Open Access articles.

500

CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		$ 82,500
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$ 82,500

Program Director/Principal Investigator (Last, First, Middle): Papautsky, Ian

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD *(from Form Page 4)*	2nd ADDITIONAL YEAR OF SUPPORT REQUESTED	3rd ADDITIONAL YEAR OF SUPPORT REQUESTED	4th ADDITIONAL YEAR OF SUPPORT REQUESTED	5th ADDITIONAL YEAR OF SUPPORT REQUESTED
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*	68,140	68,140			
CONSULTANT COSTS					
EQUIPMENT					
SUPPLIES	12,860	12,860			
TRAVEL	1,000	1,000			
INPATIENT CARE COSTS					
OUTPATIENT CARE COSTS					
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES	500	500			
DIRECT CONSORTIUM/ CONTRACTUAL COSTS					
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*	82,500	82,500			
F&A CONSORTIUM/ CONTRACTUAL COSTS					
TOTAL DIRECT COSTS	82,500	82,500			

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$** 165,000

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

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BUEDGET JUSTIFICATION – UNIVERSITY OF ILLINOIS CHICAGO

Personnel

Ian Papautsky, PhD (PI) is an expert in the development of microfluidic systems for precision medicine, including culture devices for modeling and treating cancer, as well as devices for rare cell isolation, such as circulating tumor cells (CTCs) in liquid biopsy for cancer screening. He is Co-Director of the NSF Center for Advanced Design & Manufacturing of Integrated Microfluidics (CADMIM). In collaboration with Drs. Shimamura and Basu, Dr. Papautsky will be responsible for the overall direction and progress of the experiments across both Aims. He will also ensure that research goals are met and carried out in compliance with appropriate laws and laboratory safety guidelines. He will directly supervise the research associate (Jian Zhou), and will also contribute to the overall development of experiments, troubleshooting, interpretation of data, and development of manuscripts for publication and patents.

Takeshi Shimamura, PhD (MPI) is an expert in the development of therapeutic strategies to overcome the acquired and *de novo* targeted-drug resistance using both *in vitro* and *in vivo* models in non-small cell lung carcinoma (NSCLC) and small-cell lung cancer (SCLC). In collaboration with Dr. Papautsky, Dr. Shimamura will be responsible for the overall direction and progress of the experiments across both Aims. Dr. Shimamura will directly supervise the research associate (Ines Pulido) on this study and will also contribute to the overall development of experiments, troubleshooting, interpretation of data, and development of manuscripts for publication and patents.

Jian Zhou, PhD (Postdoctoral Fellow) is an outstanding junior scientist in the Papautsky Lab. Dr. Zhou is an expert in microfluidic systems and was one of the key participants in the study presented under *Preliminary Data.* He is an expert in microfluidics and is knowledgeable in lung cancer cell culture and will coordinate with Dr. Shimamura's lab on culture of patient derived organoids (PDOs). He will also participate in the data analysis, presentation, and manuscript writing.

Ines Pulido, PhD (Postdoctoral Fellow). Dr. Pulido is an expert in NSCLC cell culture. She has worked in Dr. Shimamura's lab for more than 1 year and has learned PDO culture. Dr. Pulido will be responsible for the NSCLC cell line and PDO culture and integrating with the microfluidic platform in collaboration with Dr. Papautsky's lab. Dr. Pulido will participate in the interpretative aspects of the project, including data analysis, interpretation, presentation, and manuscript writing.

Materials & Supplies

$4,000/yr is requested to purchase supplies for fabrication of the microfluidic devices including silicon wafers, polymers (PDMS, PEGDA), mask plates, photoresists (SU8, SUEX), UV-cured adhesives, and other consumables such as glassware, plastic ware, and clean room ware. $1,000/yr is requested for supplies for microfluidic characterization of devices, including peristaltic pump, fluorescent dyes, fluorescent and non-fluorescent particles, disposable syringes, disposable tubes and fittings. $7,000/yr is requested for purchase of supplies associated with culture of cells and PDOs (disposable cell culture plates, serum, media and supplements, stereological pipettes, pipette tips, and disposables for the biosafety hood and incubators), the preparation of spheroids and organoids (cytospin slides, low adherence coatings and plates), their treatment (TKIs and chemotherapy drugs), and their analysis (e.g., fluorescent stains and immunofluorescent labels). $942/yr is requested to cover supplies associated with and costs of confocal microscopy and flow cytometry.

Travel

$1,000/year is requested to support travel by MPIs and project personnel to present the results of this work at high quality scientific meetings and conferences. Examples of such conferences include the International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS) and the annual meeting of the Biomedical Engineering Society (BMES).

Publication

$500/year is requested for expenses associated with publishing Open Access articles.

Program Director/Principal Investigator (Last, First, Middle): Basu, Anindita

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY	FROM	THROUGH
	6/1/23	5/31/25

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Basu, Anindita	PD/PI	0.24			126,825	2,537	621	3,158
Xie, Bingqing	post-doc	4			60,000	20,000	4,900	24,900
SUBTOTALS →						22,537	5,521	28,058

CONSULTANT COSTS	
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)* Please see next page	12,942
TRAVEL conference	1,000
INPATIENT CARE COSTS	
OUTPATIENT CARE COSTS	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)*	500

CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		$ 42,500
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$ 42,500

Program Director/Principal Investigator (Last, First, Middle): Basu, Anindita

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD *(from Form Page 4)*	2nd ADDITIONAL YEAR OF SUPPORT REQUESTED	3rd ADDITIONAL YEAR OF SUPPORT REQUESTED	4th ADDITIONAL YEAR OF SUPPORT REQUESTED	5th ADDITIONAL YEAR OF SUPPORT REQUESTED
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*	28,058	28,058			
CONSULTANT COSTS					
EQUIPMENT					
SUPPLIES	12,942	12,942			
TRAVEL	1,000	1,000			
INPATIENT CARE COSTS					
OUTPATIENT CARE COSTS					
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES	500	500			
DIRECT CONSORTIUM/ CONTRACTUAL COSTS					
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*	42,500	42,500			
F&A CONSORTIUM/ CONTRACTUAL COSTS					
TOTAL DIRECT COSTS	42,500	42,500			

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$** 85,000

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

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BUEDGET JUSTIFICATION – UNIVERSITY OF CHICAGO

Personnel

Anindita Basu, PhD (MPI) is an Assistant Professor in the Section of Genetic Medicine, Department of Medicine at the University of Chicago. Basu is trained in Physics and Systems Biology and has extensive experience in single cell genomics techonology development and applications. Basu co-invented Drop-Seq and will oversee single cell RNA-seq experiments (Drop-seq, 10x Genomics 3' scRNA-seq), library preparation, and sequencing in her laboratory. Basu will supervise post-doc, Xie on single cell transcriptome analysis. Together with Papautsky and Shimamura, she will help interpret research results and prepare manuscripts for publication.

Bingqing Xie, PhD (Postdoctoral Fellow) graduated from Illinois Institute of Technology with a Ph.D. degree in Computer Science with a Concentration in Machine Learning and Bioinformatics. Xie is a senior post-doc in Basu Labhas extensive experience in single cell RNA-seq data analysis and will analyze Drop-seq and 10x Genomics 3' scRNA-seq data, under Basu's supervision. Xie will also work with Basu and the UIC team for data interpretation and manuscript preparation.

Materials & Supplies

$12,942/yr is requested to purchase: A) Reagents and supplies for Drop-seq device fabrication, barcode bead synthesis, and enzymes for reverse transcription, PCR library prep, B) 3' RNA-seq kits from 10x Genomics, and C) Illumina sequencing. Multiple experiments will be pooled to reduce cost and boost the number of cells needed for each experiment. Cells from each experiment will be separated using computational tools.

Publication

$500/yr is requested for Open Access Journal publication fees.

Travel

$1000/yr is requested for travel to single cell genomics meetings.